Grant, E.J.

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UNIVERSITY OF ALBERTA

FACULTY OF ARTS AND SCIENCES

The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance, this dissertation on "An Investigation of Some Arsono Derivatives of Diphenyl Ether" and "An Investigation of the Bactericidal Power of Some Mono-Mercury Derivatives of Halogen Compounds of Fluorescein", submitted by Edward James Grant, B. Sc., in partial fulfilment of the degree of Master of Science.

AN INVESTIGATION OF SOME ARSONO DERIVATIVES OF DIPHENYL ETHER

and

AN INVESTIGATION OF THE BACTERICIDAL POWER OF SOME MONO-MERCURY DERIVATIVES OF HALOGEN COMPOUNDS OF FLUORESCEIN

by

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PART I

AN INVESTIGATION OF SOME ARSONO DERIVATIVES OF DIPHENYL ETHER

INTRODUCTION

There has been a great effort made in recent years to decrease the incidence of syphilis and this can be most effectively accomplished by adequate treatment early in the infection. This is an instance in which curative medicine is the best means of prevention of the disease. In other words the best way to prevent syphilis is to render the patients non-infectious in the shortest possible time. Fortunately synthetic organic chemistry has put into our hands some excellent new curative agents to bring about this result, as we shall presently see. Preventive medicine is often represented as more important than curative medicine; as a matter of fact in many cases they are so closely interwoven that it is impossible to separate them and this is pre-eminently true in the case of syphilis.

The incidence of syphilis has been much discussed. It may be conservatively stated that ten per cent of the community has a syphilitic taint. The economic

loss due to the disease from the standpoint of decreased earning power, cost of hospitalization, medical care, as well as premature death, is exceedingly difficult to estimate and we must therefore be content with the bare statement that it is enormous.

Modern Chemotherapeutic Work on Syphilis:-

This phase of the work had to wait upon the development of producing the disease experimentally in animals. Metchnikoff first succeeded in infecting apes with syphilis; later a method of infecting rabbits was devised and the rabbit has remained the principle animal for the work on experimental syphilis. It again emphasizes the tremendous importance of animal experimentation in all medical advance.

The term "chemotherapy" is somewhat difficult to define. It was introduced by Ehrlich and the definition and field of work indicated by the term can be best illustrated by the work done in Ehrlich's laboratory on syphilis and trypanosomiasis. Chemotherapeutic work on syphilis was an outgrowth of the work on trypanosomiasis, and for this reason we must briefly discuss trypanosomiasis at this time. Under the term "trypanosomiasis" we include a series of diseases of man and animals which are produced by organisms

related to the organism causing syphilis. These diseases such as African sleeping sickness are of tremendous importance in the world and will be referred to later on in this paper. Trypanosomal infections are of importance in connection with the chemotherapy of syphilis because they can be given to animals very readily, and the animals die promptly unless effective remedies are administered. Thus, the duration of life in rats in many forms of trypanosomiasis is only three to nine days, whereas experimental syphilis runs an extremely chronic course.

Ehrlich's views regarding chemotherapy were based on the general proposition that certain organic chemicals have a specific affinity for certain living cells, and the term was an outgrowth of Ehrlich's views regarding the nature of immunity. We would define chemotherapy as that phase of pharmacology which deals with the relation between the chemical structure of therapeutic agents and their effect on living things. Ehrlich used the term only in reference to infectious diseases but there is no reason why it should not include all types of substances used in therapeutics.

In practice, work in this field has always started with a fortuitous discovery that a given organic

chemical has a certain specific pharmacological action. Following this lead, various derivatives of the drug are then prepared in the hope of finding a substance which is less toxic to man and more potent in the desired type of pharmacological activity than the original substance. The production of derivatives is not done in a routine or haphazard manner. We have accumulated a certain body of fact regarding the biological effect of certain groupings and the derivatives are made in accordance with past experience. In the case of the materials used in the treatment of infections, chemotherapeutic studies are directed towards the production of substances highly toxic to invading parasites and of low toxicity to man and the higher animals. The whole field of chemotherapy is one of the most promising for medicine of the future. It is on the border-line between chemistry and medicine and requires the cooperative effort of chemists, bacteriologists, pharmocologists and clinicians. The particular phase of chemistry concerned in most cases is synthetic organic chemistry and the work must be carefully coordinated in order to be successful.

The Earlier Arsenicals:-

In 1863[(1)] Béchamp prepared a substance by the action of arsenic acid on aniline which he thought

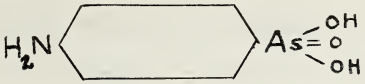
to have the formula, $C_6H_5NH.\ AsO\ (OH)_2$. He also prepared several salts of this acid. The sodium salt was first used therapeutically by[2] Schild and Kionka in 1902 and the first animal experimentation with the substance was done by[3] Blumenthal in the same year. They were interested in the sodium salt of this acid from the standpoint of general arsenical therapy, and not from the standpoint of the treatment of syphilis or trypanosomiasis. Blumenthal found it much less toxic to animals than the inorganic forms of arsenic and this relatively low toxicity caused the adoption of the name "Atoxyl" for the substance.

The early literature of the last century contains references to the clinical use of arsenic in inorganic form in the treatment of trypanosomiasis. Laveran and Mesnil, in 1902, studied the action of inorganic arsenical compounds in experimental trypanosomiasis and found them somewhat effective in that they checked the infection but never cured the disease. (4)Thomas in 1905 first studied the action of atoxyl in experimental trypanosomiasis and found it to be an effective curative agent. [5]Robert Koch in 1907 showed that in clinical trypanosomiasis, atoxyl causes the organisms to disappear from the blood stream, and from that time

up to the present atoxyl has continued to be used clinically in the treatment of trypanosomiasis in the human.

(6) Ehrlich began his chemotherapeutic studies on the arsenic compounds with atoxyl. In the beginning he studied the effect of atoxyl on trypanosomes in the test tube and found it was not effective; even in high concentrations it did not kill the organisms. For this reason he temporarily discontinued work with it. Later Ehrlich again studied the action of atoxyl in the living animal infected with trypanosomes and confirmed the observations of Thomas that it is very effective. Several investigators have shown that blood or tissue extracts, when treated with atoxyl, yield a substance,that which kills trypanosomes in the test tube. It was evident therefore that atoxyl has to be altered in the body before it is capable of destroying these organisms. Following the atoxyl lead, Ehrlich and his co-workers prepared a very large number of derivatives of atoxyl. So far a complete list of these substances prepared and studied by them has never been published. Ehrlich and Bertheim later made a study of the structure of atoxyl and found that instead of being of the nature of an anilide, it is

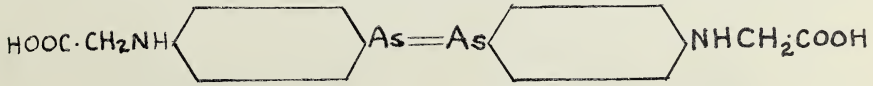
really para-amino - phenylarsonic acid



In their monograph on chemotherapy Ehrlich and Hata mention specifically relatively few compounds. It soon became evident from clinical work on atoxyl that the drug produces serious injury to the eyes in a considerable number of cases, occasionally causing complete and permanent blindness. The first attempt to modify atoxyl and to reduce its toxicity resulted in the production of its acetic acid derivative, arsacetin,

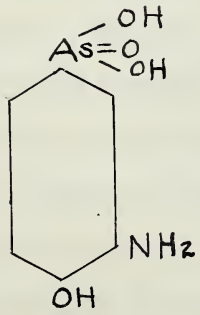
This substance continued to be used clinically in the treatment of trypanosomiasis for many years, although it had just as severe an effect on the optic tract as atoxyl and possessed no advantages over it.

Among the large number of derivatives produced by Ehrlich and very extensively studied by him and his co-workers was arsenophenylglycine

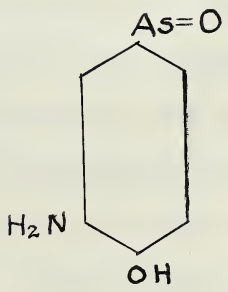


Ehrlich regarded this substance as highly satisfactory in the treatment of trypanosomiasis. The basis for this statement was that animals were cured of trypanosomiasis by a single treatment with the drug.

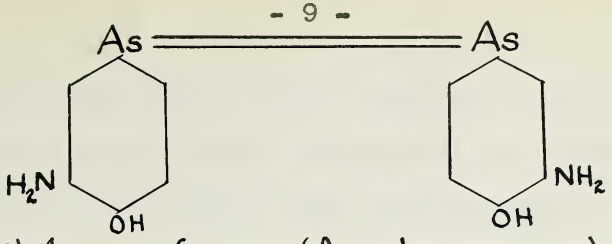
Arsphenamine (Salvarsan) or "606" - Ehrlich had the dream of "therapia sterilisans magna" - ie., a drug which would produce a complete sterilization in a single dose both in syphilis and trypanosomiasis. In regard to syphilis, certainly this dream has never been realized, and in regard to trypanosomiasis it has only been realized in the case of the smaller animals such as the mouse, rat and rabbit. There are three types of organic arsenicals which have been studied especially in syphilis and trypanosomiasis. They are (1) the arsonic acid, (2) the arseno and (3) the arsinoxide types. All three types of compounds may be illustrated with the arsphenamine nucleus, as follows:



(1) Arsonic Acid



(3) Arsinoxide



(2) Arseno form (Arsphenamine)

Arsphenamine was finally accepted by Ehrlich as the most favorable of all the compounds which he studied in the treatment of syphilis; no subatance approaching it in value has been since discovered. Ehrlich was so pleased with its effects and so convinced that the new drug would be the salvation of mankind by destroying one of its greatest scourges that he named the drug "salvarsan"; it also became known as "606" under which name it was recorded in Ehrlich's laboratory in the long list of arsenicals prepared for study. Its pharmacopoeial name is arsphenamine. The substance was placed on the market in vacuum sealed ampules in the form of the dihydrochloride in 1910. Very remarkable clinical effects were reported at once and the discovery startled the world; it was thought that this dread disease had been vanquished by one stroke. Many cases of syphilis with most distressing lesions, which had received mercury and iodides, yielded immediately to arsphenamine and healed. It was soon found, however, that one treatment could not cure and that it requires great persistance to effect a cure of syphilis

in man in most cases. This compound is readily soluble in water but on account of its acidity is unusable in this form. Four equivalents of caustic soda must be added before it is ready for injection into the vein in the form of a solution of the di-sodium salt. To inject the uneutralized material into the vein would cause almost instant death.

The most striking improvements in arsphenamine have been in methods of making it more easily soluble. Thus Ehrlich's laboratory also produced neoarsphenamine

$$NaOSOCH_2HN.C_6H_3(OH)As{=}AsC_6H_3(OH)NHCH_2OSONa$$

a product of the action of sodium formaldehyde sulph-oxylate on the amino group of arsphenamine. It is a great improvement from the standpoint of convenience to the physician because it is unnecessary to neutralize the product prior to injection. The contents of the ampule are simply dissolved in distilled water and may be injected directly into the vein with the use of a hypodermic syringe. Neoarsphenamine can also be given in a more concentrated solution than arsphenamine. It has certain advantages and certain disadvantages. It is less toxic than arsphenamine and less likely to produce severe reactions. It also has the advantage that it effects a more prompt healing of active

syphilitic lesions. Apparently the reason is that the substance is less colloidal, it penetrates into the tissues more readily, and it seems to increase the resistance of the tissues to the presence of the treponema pallidum. Its disadvantages are that it is less treponemicidal; it is more unstable chemically; it is not such a definite chemical substance on account of the indefiniteness of the formaldehyde sulphoxylate group, and a larger dose is required than of arsphenamine, although the margin of safety, i.e. the therapeutic index, is at least as great.

Ehrlich was the first to introduce the term "therapeutic index" and by this term he meant the ratio of the toxic dose to the therapeutic dose. It should be pointed out that the therapeutic index as determined from experimentation with small animals often does not hold for man and the larger animals. Thus Bayer 205 a non-arsenic containing product offered for sale under the name of "Germanin", has been a great disappointment in the treatment of human trypanosomiasis in spite of the fact that it has a very high therapeutic index in trypanosomiasis in rats, but it is of no value in the treatment of human trypanosomiasis.

Another closely related derivative of neoarsphenamine is a preparation called sulpharsphenamine pro-

duced by [7] Voegtlin, Johnson and Dyer in the Hygienic Laboratory of the U. S. Public Health Service. It has the following structure:

$$NaO_2SOCH_2NH.C_6H_3(OH)As = As\,C_6H_3(OH).NHCH_2OSO_2Na$$

This product although very similar chemically to neoarsphenamine, differs from it somewhat in its biological effects and may have advantages over it in the treatment of certain syphilitic lesions.

As stated previously, Ehrlich observed that atoxyl does not destroy trypanosomes in the test tube. He also observed that the arsphenamines are not effective in the test tube. On the other hand, the arsinoxide compounds corresponding to these substances are effective outside the body. Voegtlin and Smith showed that when the arsonic acids or the arsphenamines are injected into rats whose blood swarms with trypanosomes there is a certain latent period varying from one to three hours before the trypanosomes begin to disappear from the blood. On the other hand when the arsinoxides are administered the trypanosome count begins to be reduced at once. They very logically conclude from this work that both the arsonic acid and the arseno types of compounds have to be changed in the body into the corresponding arsinoxides before

they become effective. The latest period observed before the organisms begin to disappear can be most readily explained as due to the time required to build up an effective concentration of the arsinoxides. It has been found by all investigators who have studied the question, that in therapy the arsinoxides cannot be used as successfully as the other types of compounds. The relative parts played by the parasitic organisms and by the cells of the host in the reduction of the arsonic compounds, or in the oxidation of the arseno compounds, may account for the fact that the drugs can best be administered in the inactive forms and permit them to be activated by the living body.

Stovarsol is the acetyl derivative of the arsonic acid corresponding to arsphenamine. It has the formula: $CH_3CONH.\ C_6H_3\ (OH).\ AsO\ (OH)_2$ It was prepared by Ehrlich and his collaborators. This drug has been used extensively in France in the treatment of syphilis and is undoubtedly far less effective than the arsphenamines. The increase in syphilis which has bean noted in France during the last few years has been attributed by some writers to the fact that Stovarsol has been very largely substituted for the arsphenamines, with the result that the patient

remains longer in a state in which he can infect others.

It seems highly probable that other drugs might be found which would be more effective than arsphenamine in the treatment of active syphilis. The drugs sometimes produce very disagreeable and occasionally even fatal disturbances and, in the opinion of the writers, work should continue in an effort to find still more effective drugs in the treatment of the various manifestations of acute syphilis.

Syphilis of the Central Nervous System

General Paresis - Tryparsamide. General paresis is one of the most distressing and fatal forms of mental disease. In the treatment of this condition, the arsphenamines proved themselves to be of very little value. In a systematic study of the chemotherapy of neurosyphilis by Lorenz, Loevenhart, Bleckwenn and Hodges, it was determined to study the action of drugs in the treatment of this condition irrespective of their effectiveness in the earlier stages of syphilis. Among the substances studied by them was tryparsamide,

$$H_2NCOCH_2\ NH.\ C_6H_4\ AsO\ (OH)_2$$

This substance is the amide of the arsonic acid which had been previously studied by Ehrlich in the arseno form; viz., arsenophenyl glycine. It was first

produced at the Rockefeller Institute for Medical Research by Jacobs and Heidelberger and studied experimentally in animals by[8] Brown and Pearce in the same Institute. It was found by Lorenz and his coworkers that drug is exceedingly effective in the treatment of general paresis and resulted in the mental restoration of 40% of the patients who had been committed to state institutions, enabling them to return to their families and to resume their work, often with increased earning capacity. This important result has been comfirmed by a large number of investigators. Tryparsamide has a remarkable tonic effect and the patients frequently gain from ten to thirty pounds in weight during the treatment.

Tryparsamide also has the disadvantage that in one to two per cent of the cases, especially where there is involvement of the spinal cord there occurs a more or less serious impairment of vision which has to be carefully watched. It is in this regard in particular that there is a necessity for finding a better drug than tryparsamide.

Other Drugs Used in the Treatment of Syphilis.

Mercury and iodides continue to be valuable adjuncts in the treatment of certain types of syphilis.

There has been no great improvement in the use of these drugs. A great deal of work has been done on the chemotherapy of mercurial compounds but no practical advance has thus far been made, although the synthesis of new mercurial compounds offers a very promising field in the treatment of syphilis. Various compounds of bismuth have recently been introduced and they are undoubtedly of value in the treatment of syphilis, especially in cases which have a peculiar sensitiveness to the arsphenamines but bismuth compounds are by no means as effective as the arsphenamines. It is possible that bismuth compounds may in the future replace mercury to a certain extent.

The arsphenamines have been found to be exceedingly useful in the tropical disease known as frambesia or yaws , which is very prevalent in certain districts. It seems as though this disease could be completely wiped out by the extensive use of arsphenamines. Arsphenamine is also valuable in the treatment of relapsing fever and has also yielded favorable results in the treatment of Vincent's angina. The organisms responsible for these conditions are more closely related to treponema pallidum than to trypanosomes.

Trypanosomiasis - African Sleeping Sickness - Tryparsamide

Trypanosomiasis still presents a great world problem. Approximately one million square miles of equatorial Africa is affected by trypanosomal diseases, which in man cause the African form of sleeping sickness, and in animals cause the disease known as nagana. African sleeping sickness is an entirely different disease from encephalitis lethargica - the so-called "sleeping sickness" occurring in this country. The African disease has at certain periods caused the majority of deaths in certain regions and has decimated the population. It also attacks all of the domestic animals. Tryparsamide was shown by Brown and Pearce to be exceedingly effective in the treatment of experimental trypanosomiasis, and Pearce showed that it is the most beneficial drug known in the treatment of human trypanosomiasis, especially in the later stages where there is involvement of the central nervous system. Investigators have found tryparsamide to cause the same remarkable mental restoration that had been previously noted by Lorenz and his co-workers in general paresis. The work of Pearce has been amply confirmed and there is no doubt that it represents the greatest advance in the treatment of human trypanosomiasis that has been made thus far. Here again it has been observed that tryparsamide, in a certain percentage of

the cases, deleteriously affects the vision, and it is especially from this standpoint that further work needs to be done to develop drugs which are safer from this view-point. It has been found that tryparsamide is not so effective in the treatment of the form of South African sleeping sickness caused by Trypanosoma rhodesiense. The more common form which yields to tryparsamide is caused by Tr.gambiense. The drug has also been found to be quite ineffective in the treatment of certain forms of trypanosomal disease attacking horses and cattle, such as the disease known as surra, which is prevalent in the Philippine Islands and causes enormous economic loss.

Experimental trypanosomiasis, it is seen, has played a great role in chemotherapeutic studies of syphilis. It should be pointed out, however, that there is by no means a parallelism between these two diseases; since drugs are known which are very effective in syphilis and totally inert in trypanosomiasis, and the converse is also true.

Only the briefest outline of the combat against syphilis, has been given, but it shows a most hopeful outlook for ultimate victory over the disease and, in time, possibly its complete suppression. This great

advance in the treatment of the disease would not have been possible without the aid of creative chemistry. So great indeed have been the results of the co-operation of medicine and chemistry in this field that the workers against other diseases have been heartened and encouraged to follow the plan of campaign that was initiated by Ehrlich.

Pharmacological Action:

The mechanism of the pharmacological action of these drugs has been extensively studied. Ehrlich noted atoxyl and arsphenamine did not destroy parasites in vitro but the corresponding arsinoxide compounds were effective. Voegtlin and Smith[9] showed that there is a latent period after injection of arsonic acids or arsphenamines before the trypanosomes begin to disappear from the blood of the affected animal. On the other hand when the corresponding arsinoxides are administered the trypanosome count begins to be reduced at once. The logical conclusion therefore is that the arsonic acids and arseno types of compounds have to be changed in the body to the arsinoxide form before they become effective. The latent period is due to the time required to build up an effective concentration of the arsinoxides. However, investigators have shown

that the arsinoxides cannot be used as successfully as the other types of compounds in therapy. The organisms or cells of the host probably play a specific part in the reduction of arsonic acid compounds or oxidation of arseno compounds and this may account for the fact that drugs can be best administered in the inactive forms and activated in the living body.

Voegtlin[10] supposes the arsinoxide derivative once it is formed reacts with reduced glutathione of the tissues.

$$R.As=O \begin{matrix} HS-G \\ HS-G \end{matrix} \longrightarrow R.As \begin{matrix} S-G \\ S-G \end{matrix} + H_2O$$

Trypanosomes have been shown to contain -SH groups (nitroprusside test) similar to those in reduced glutathione. Voegtlin's hypothesis apparently assumes that trypanocidal action is due to a combination of the arsinoxide with these sulfhydryl groups. In other words the -SH group acts as a chemoreceptor for the arsenic.

In the more recent work on organic arsenical compounds Cohen, King and Strangeways[11] have contributed a considerable part. Their work on the velocity of oxidation of arylarsinoxides offers an explanation for the variation of the therapeutic effect of the different arsonic acids. They consider the therapeutic

value of different arsonic acids may depend on the speed of reduction of the pentavalent acid to the trivalent arsinoxide. Their work has been done in two main directions: (a) the determination of oxidation-reduction potentials of various arsonic acids - arsinoxide systems, (b) studies of velocities of oxidation of various arsinoxides. The former method considers the arsonic acid-arsinoxide system similar to the ferrous-ferric iron system, but up to the present time it has failed to yield definite results. However, considerable progress has been made with the velocity of oxidation method. The determination of the velocity of reduction would be a more direct method but it involves a lot of experimental difficulties. The oxidation method is feasible and optically active cystine is used as the oxidizing agent.(12)

$$R.As\begin{matrix}OH\\OH\end{matrix} + \begin{matrix}S - CH_2CH(NH_2)COOH\\S - CH_2CH(NH_2)COOH\end{matrix} \xrightarrow{OH'}$$

$$R.AsO_2 + 2\,SH\cdot CH_2CH(NH_2)COOH$$

The arsenicals can be classified by their ease of oxidation, according to the bimolecular velocity constants. Cohen and his co-workers assume that the increasing order of velocities of oxidation of a series of arsinoxides towards a common oxidizing agent is the reverse of the order of the velocities of reduction of

the corresponding arsonic acids, considering the arsonic acid - arsinoxide system is reversible. They follow the reduction of cystine polarimetricalling the specific rotation of cystine changing from 104° to -3° as cystine is reduced to cysteine.

They found that as primary arsonic acids are reduced to arsinoxides the toxicity to the host is greatly increased. The toxicity to the host may be used as a measure of the amount of reduction by the tissues since reduction must take place before the compound can exert trypanocidal activity. It is found however, that the direct administration of the arsinoxides doesn't give rise to the same toxic effects as the use of the corresponding arsonic acids. Other factors such as the rate of excretion may mask the relationship between the ease of reduction and toxicity. Voegtlin and Smith[13] showed that 80% excretion of arsonic acids from rats occurred in 6 hours. Thus the amount of arsonic acid available for reduction in the tissues must be a very small percentage of the amount administered. Relationship between the ease of reduction and curative action is not easily discernible since curative action is measurable only within the limits of non-toxicity to the host. Cohen, King and

Strangeways conclude that neither from the curative action nor the toxicity to the host can any deduction be made as to the rate of reduction in the tissues. This rate is only one of the many factors determining the activity.

Sulphur Derivatives:[14] The remarkable affinity of arsenic for organically linked sulfur and the fact that such sulfur occurs in the tissues, lends considerable interest to the study of thiolarsenic compounds. Thioarsenites are substances with trivalent arsenic linked through sulfur with organic radicals. The first compound of this type to be prepared was, arsenic trithiolacetic acid $As(SCH_2COOH)_3$ by Rosenheim and Davidsohn[15]. Friedberger[16] obtained a compound with trypanocidal activity from a reaction postulated to be as follows by Bertheim[17].

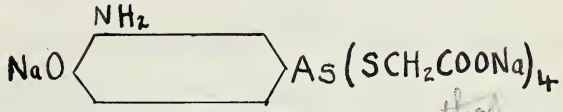
$$R.AsO_3H_2 \xrightarrow{SHCH_2COOH} R.As=O \xrightarrow{SHCH_2COOH} R.As\begin{matrix}SCH_2COOH\\SCH_2COOH\end{matrix}$$

The product is di(carboxymethyl) 4-amino-phenylthioarsenite. Kharasch[18] patented a method for the preparation of water soluble organo-metallic compounds according to the reaction,

$$Ar.AsCl_2 + 2\,SHCH_2COOK \rightarrow Ar.As\begin{matrix}SCH_2COOH\\SCH_2COOH\end{matrix}$$

Trivalent arsenicals are insoluble in water and behave as weak acids. Condensation with thiol compounds

containing carboxyl groups made such oxides effective for uses similar to those of pentavalent arsenicals. In 1928 Parke Davis and Co. were granted a patent[19] for new pentavalent derivatives which were claimed to be increased in toxicity to spirochaetes. They were of this type:



Voegtlin, Dyer and Leonard[20] observed that tervalent arsenicals in the same way produced compounds with less toxicity than the parent compound.

In 1929 Barber, May and Baker Ltd.[21] patented a process for the preparation of thioarsenite derivatives from one mole of arsonic acid and four moles of thiol compound (2 moles used for reduction and 2 moles for condensation):

$$Ar{\cdot}As\leqslant\begin{matrix}OH\\OH\end{matrix}O + 4RSH \rightarrow Ar{\cdot}As\leqslant\begin{matrix}SR\\SR\end{matrix} + R\text{-}S\text{-}S\text{-}R + 3H_2O$$

The thiol group of thiol acetic acid for example reduces the pentavalent arsonic acid to the trivalent state and the product reacts with the excess reagent to the compound produced above.

Barber[22] also introduced thiolacetamide as a reagent for the indentification of arsonic acids according to the reaction:

$$Ar{\cdot}As\leqslant\begin{matrix}OH\\OH\end{matrix}O + 4\,SHCH_2{\cdot}CO{\cdot}NH_2 \longrightarrow 3\,H_2O +$$

$$Ar{\cdot}As\leqslant\begin{matrix}SCH_2{\cdot}CO{\cdot}NH_2\\SCH_2{\cdot}CO{\cdot}NH_2\end{matrix} + H_2N{\cdot}CO{\cdot}CH_2\text{-}S\text{-}S\text{-}CH_2{\cdot}CO{\cdot}NH_2$$

The di(carbamylmethyl) arylarsenite produced has a definite melting point.

Barber[23] reported no hydrolysis of thiolarsenites by alkali and got an intense nitroprusside test from the intact thioarsenites.

Cohen, King and Strangeways[14] found a partial hydrolysis in weak alkali or aqueous solutions with a weak or negative nitroprusside test in a $NaHCO_3$ solution whereas the parent thiol aliphatic compounds give a strong test. Solution in N/10 NaOH results in an intense nitroprusside test. This infers the solutions of thioarsenites have the intact molecule in equilibrium with the hydrolytic products. With strong alkali there is considerable hydrolysis but little with $NaHCO_3$ as follows:

$$R{\cdot}As\lessdot^{SR'}_{SR'} + 2H_2O \rightleftharpoons^{OH'}_{H^+} RAs(OH)_2 + 2RSH$$

These workers observed alkaline cystine and an arsinoxide develops an intense nitroprusside reaction indicating cysteine:

$$R{\cdot}As=O + \begin{matrix} S-R' \\ | \\ S-R' \end{matrix} + 2H_2O \xrightarrow{OH'} R{\cdot}AsO_3H_2 + 2HSR'$$

They say the inability of thiol compounds and arsinoxides to condense and thiol compounds to reduce arsonic acids in alkaline solution invalidates Barber's mechanism (loc.cit.). In solutions near neutrality an

arsonic acid combines with four moles of the thiol compound forming a tetrathioarsonate, as an intermediate and then two thiol alhyl groups are split off to link with each other:

$$RAsO(OH)_2 + 4\,HS\cdot R' \longrightarrow R\cdot As(SR')_2 + (\cdot SR')_2$$

The intermediates have not been isolated as yet, thus the Parke Davis tetrathioarsonates are really mixtures of thioarsenites and disulfides. They postulate also the reduction of a disulfide by an arseno compound in alkaline solution.

$$\begin{matrix} R\cdot As \\ \| \\ R\cdot As \end{matrix} + 2\,\begin{matrix} SR' \\ | \\ SR' \end{matrix} \longrightarrow 2\,R\cdot As\begin{matrix} SR' \\ < \\ SR' \end{matrix}$$

Therapeutic Results: Rosenthal and Voegtlin[24] showed glutathione inhibits the toxic action of an arsinoxide on trypanosomes in vitro in the proportion of 10:1 of glutathione to arsinoxide. Glutathione combines with the arsinoxide forming a dithioarsenite and the excess reducing the dissociation of the complex to a minimum. This supports the claim that the toxic activity of thioarsenites on trypanosomes is not a property of the intact molecule but of the hydrolytic product, arsinoxide.

Cohen and his colleagues summarize the theory on the mechanism of the lethal action of arsenicals on trypanosomes. Ehrlich[25] expressed the view that

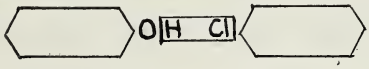
the toxic action of arsenicals might lie in their affinity for the thiol groups of tissues. Voegtlin has also adopted and expanded this view regarding glutathione as the main arsenic-receptor and considers the reaction with glutathione interferes with the respiration of the living cells.

In conclusion, the view of Cohen, King and Strangeways[14] is that the remarkable affinity of arsenic for organically bound sulfur as demonstrated in their experiments justifies the hypothesis that lethal action of arsenicals on living tissues is a chemical action on thiol groups especially occurring in glutathione.

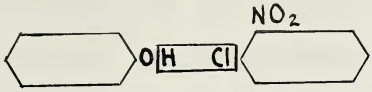
Outline and Discussion of the Investigation

Because of the importance of the diphenyl ether group in such compounds as thyroxyin and its derivatives it was thought that this group may have a marked chemotherapeutic effect, especially when coupled with the arsono group. Furthermore diphenyl ether is now readily available as an industrial by-product. Up until the present time no work has appeared in the literature on the preparation of arsenic derivatives of diphenyl ether.

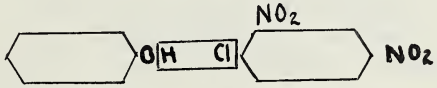
The starting point in the preparation consists in a coupling reaction between a nitroarylhalide and the potassium salt of a phenol. The reaction,



is extremely difficult to carry out but a reaction such as

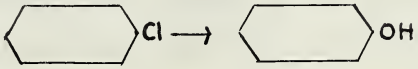


or

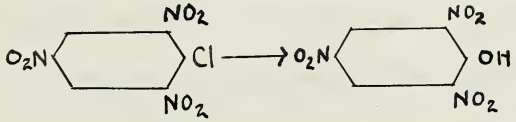


is readily carried out. When a nitro group is present in the arylhalide in the ortho or para position the reaction goes on readily due to the activating effect on the halogen by the nitro group.

For example:



This reaction proceeds only in a solution of NaOH at a temperature of 350°. But with picryl chloride the hydrolysis to picric acid can be accomplished by hot water.



According to the theory of Kharasch[26] the introduction of the nitro group in the ortho or para position to the halogen tends to make the phenyl group less electronegative. The strength of the bond

between the halogen and the phenyl group is weakened due to the shift of the pair of bonding electrons away from the phenyl group. It follows that because of this weakening the hydrolysis reaction takes place much more readily.

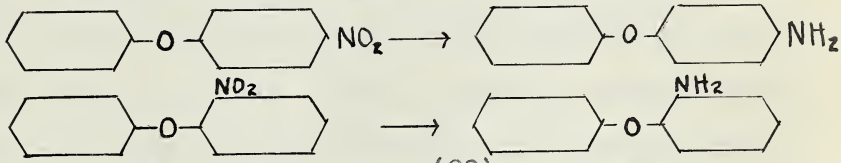
The following ethers were prepared by means of a condensation such as the one described above:

4 - nitrodiphenyl ether
2 - nitrodiphenyl ether
2,4, - dinitrodiphenyl ether.

The 2,4 - nitrodiphenyl ether was synthesized according to the method described by Raiford and Colbert[27] in 1926. Nitrochlorobenzene was added to a potassium phenolate solution. The phenolate was prepared by adding the alkali to an aqueous solution of phenol. The mixture was then refluxed on an oil bath at 150° for several hours. A large excess of phenol was used and so a negligible amount of nitrochlorobenzene was left. Thus there was no necessity for steam distillation to remove the latter. Alkali was added to remove the excess phenol from the reaction solution and the ether crystallized out at this point. The supernatant liquid was decanted off and the residue washed with water. The ether was recrystallized from ethyl alcohol.

For the preparation of the 2 and 4, nitrodiphenyl ethers a slightly different method was used following the procedure reported by Henley[28]. The method was very similar, but 1 mole of the chloronitrobenzene to 1.75 moles of phenol and 1.25 moles of potassium hydroxide were condensed. Only 0.1 mole of water was used and the mixture was heated for one hour at 200 - 210°. The ethers were recovered in the same way as above.

The next stage was the reduction of the nitro groups attached to the ethers.



The procedure described by Suter[29] in 1929 was used. The reducing agent was zinc dust and dilute ethyl alcohol in a slightly acid medium. Calcium chloride was used to adjust the acidity. This reduction works very smoothly and good yields were obtained. The mechanism of the reaction is as follows:

$$Zn \longrightarrow Zn^{++} + 2\ominus$$

$$-N\overset{O}{\underset{O}{\leq}} + 6\ominus \longrightarrow -N\overset{H}{\underset{H}{<}}$$

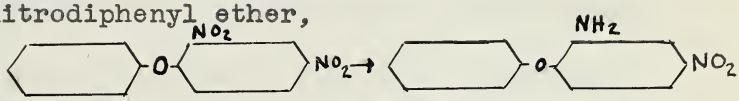
The hydrogen atoms required were supplied by the reaction of the zinc with the water in this manner:

$$3Zn + 6H_2O \longrightarrow 3Zn(OH)_2 + 3H_2$$

$$\hookrightarrow ZnO$$

The reaction was carried out on a steam bath and the amine was recovered by diluting the solution.

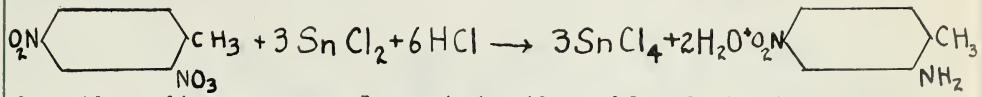
A different procedure was necessary to reduce the 2, 4 - dinitrodiphenyl ether,



The reducing agent in this case was stannous chloride in alcohol saturated with hydrogen chloride. This reaction although appearing fairly simple presented a great deal of difficulty and a considerable loss of time. Using a solution with a low hydrogen ion concentration resulted in nothing but a tarry mass. Attempts were made also to use acetic acid as a solvent but met with no success. With a greater concentration of acid and using stannous chloride in molecular proportions good results were obtained. The stannous chloride solution was added slowly from a dropping funnel to the dinitro ether dissolved in alcohol. The temperature of the reaction was kept down using a cold water bath and the mixture was stirred constantly. Alkali was not required to liberate the amine; it was found that just by dilution with water the salt was hydrolysed and the amine separated out.

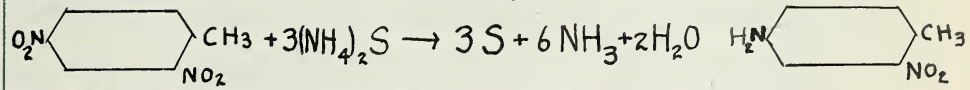
This reduction reaction is especially interesting, since it is an excellent example of the specificity

of stannous chloride as a reducing agent in the stepwise reduction of polynitro compounds. The earliest known example of this property of stannous chloride, is the reduction of dinitrotoluene in this manner:



ie. the nitro group closest to the side chain is reduced while the other nitro group remains unattacked. Similarly in the case of 2, 4 - dinitrodiphenyl ether the nitro group closest to the phenoxy radicle is the one to be reduced.

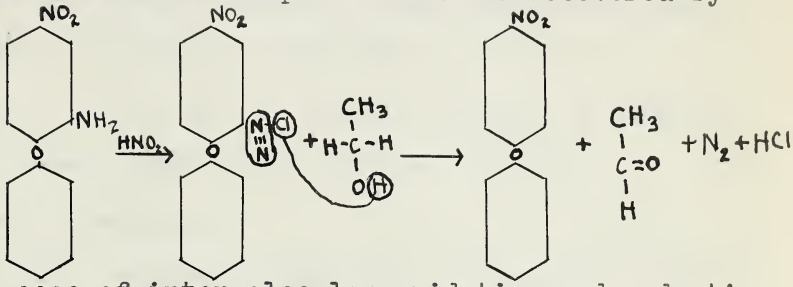
Another good stepwise reducing agent specific in its action is ammonium sulfide,



This ammonium sulfide has selective action on the nitro group farthest removed from the side chain. It would appear highly probable that this reducing agent has the same action on the 2, 4 - dinitrodiphenyl ether, reducing the nitro group in the 4 position.

The structure of 2, 4 - dinitrodiphenyl ether was investigated and the proof of the amine group being in the 2 position was verified in two different ways. First the amino group was removed and the nitro compound compared with a similar compound prepared by direct condensation. The amino group was diazotized

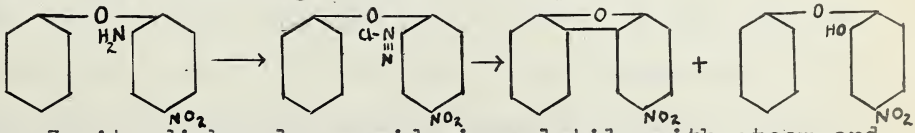
in alcoholic solution, in the presence of sulfuric acid. The solution was then warmed until no more gas came off and the nitro compound then was recovered by dilution.



This is a case of intermolecular oxidation and reduction.

It was found 4 - nitrodiphenyl ether prepared by the condensation of potassium phenolate with nitrochlorobenzene had a melting point 57°. The ether prepared by alcoholic diazotization had a melting point of 58°. A mixed melting point determination gave a result, 58°. This is direct proof that the amino group is in the 4 position.

For further verification a diphenylene oxide was prepared. The nitro amino ether was diazotized and the diazonium solution was poured into hot 50% sulfuric acid. The following reaction took place:



The 3-nitrodiphenylene oxide is volatile with steam and was removed by steam distillation. This is a standard procedure for the preparation of substituted diphenylene

oxide compounds.

The next step in the procedure was the introduction of the arsono radicle. This could have been accomplished in either of two ways:

(1) Directly by the use of arsenic acid, in a similar manner to direct nitration, sulfonation, etc.

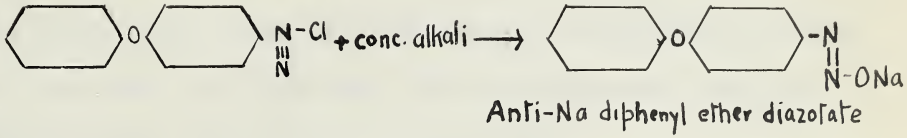
(2) By Bart's Reaction.

The latter is a general method for synthesizing aromatic arsono compounds developed by Bart[30] in 1912. The amine is diazotized, coupled with sodium arsenite and warmed to remove the nitrogen gas. Metallic salts in an alkaline solution catalyse the reaction.

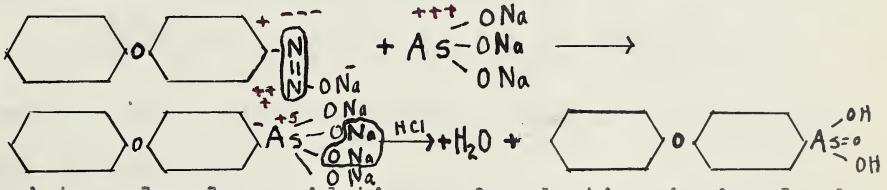
$$R\cdot N{=}N{-}Cl + Na_3AsO_3 \longrightarrow R\cdot As{=}O{<}^{ONa}_{ONa} + NaCl + N_2$$

The method used was a modified form of the method described by Saunder and Hamilton[31]. The nitro amino compound was dissolved in hydrochloric acid and diazotized. The cold diazonium solution was poured into a solution of sodium arsenite in concentrated alkali. Copper sulfate was used as a catalyst and the mixture was kept cold by the addition of ice. It was stirred vigorously until the evolution of gas ceased and then allowed to stand for several hours to insure complete coupling. The solution was then warmed and filtered and the arsono compound liberated by the addition of hydrochloric acid. Since the coupling takes place in

concentrated alkali the anti-diazotate must be formed. This is contrary to coupling reactions in general, where the best coupling takes place with the syn isomer in the presence of dilute alkali. The mechanism of this reaction is as follows:

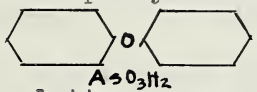


Anti-Na diphenyl ether diazotate

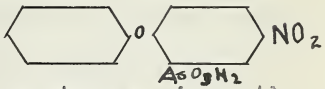


An intermolecular oxidation and reduction is involved. The arsenic atom is oxidized thus,

and the terminal C atom on the ether is reduced. Similarly 2 - arsonodiphenyl ether and 2 - arsono - 4 - nitrodiphenyl ether were prepared.



and



The latter compound was of great importance since the nitro group could be reduced to an amino group. It is the arsono amino grouping which causes the therapeutic activity in the various synthetic organic arsenic compounds. The reduction of the nitro group caused considerable difficulty because it was difficult to prevent the reduction of the arsono group also, to an arseno compound or an arsinoxide.

A series of reducing agents were tried:

(1) Alkaline ferrous chloride.

(2) Alkaline glucose.

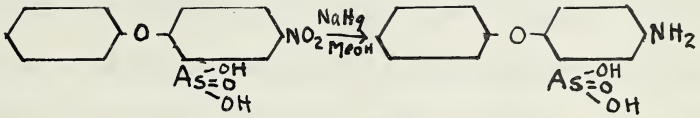
(3) Sodium hydrosulfite.

(4) Sodium amalgam.

The first two reducing agents gave no definite results and (3) was too vigorous. It probably gave the diphenyl ether derivative of salvarsan

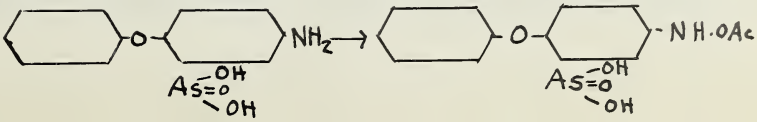
$$C_6H_5O{\cdot}H_2N\,C_6H_3{\cdot}As=As{\cdot}C_6H_3{\cdot}NH_2{\cdot}OC_6H_5$$

However, using sodium amalgam a successful reduction was carried out,



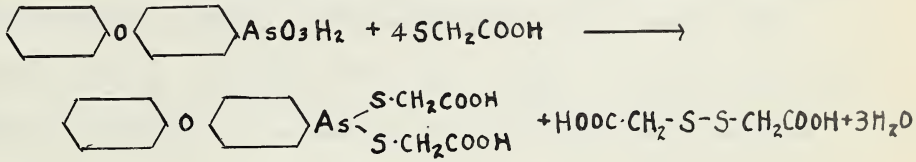
The arsono compound was dissolved in methyl alcohol and a 4% amalgam was added carefully with shaking and cooling. The solution was decanted from the mercury and the alcohol distilled off till a small volume remained. Water was added and the solution filtered. It was made acid with hydrochloric acid and again filtered. Alkali was added to the filtrate until a maximum precipitate appeared.

An attempt was made to acetylate the amino group in order to lower the toxicity of the compound.



This was done by refluxing the amino compound with acetic anhydride but the attempt was not successful.

The arsono compounds can be readily characterized by a method reported by Barber[22] for preparing solid crystalline derivatives with definite melting points, using thiolacetic acid as a reagent. The derivative was prepared according to this reaction in a $NaHCO_3$ solution:



Four moles of thiolacetic acid are required for this reaction; two moles reduce the pentavalent arsenic to the trivalent state and the excess thiol compound combines with the trivalent arsenic to give the derivative. The di(carboxymethyl) diphenyl ether thioarsenite is crystallized from dilute acetic acid and can be analysed by direct titration with standard iodine solution.

$$Ar\cdot As(SR)_2 + 3H_2O + 2I_2 \rightarrow ArAsO(OH)_2 + 4HI + R\text{-}S\text{-}S\text{-}R$$

Because of the difficulty in getting this type of compound to crystallize the above compound was the only one of its kind prepared. It may be noted by reversing the procedure using a known arsono compound

this method would serve for indentifying mercaptans.

The method used for the estimation of the arsenic in the various compounds was the one described by Morgan[32]. A small quantity (.1-.2g) was washed into a 300 cc. Kjeldahl flask. Ten grams of potassium sulfate and .2g of starch was added. Twenty cc. of concentrated sulfuric acid was next cautiously added and the mixture digested, a low flame being used until the frothing ceased. The digestion took about four hours and was complete when the liquid turned colorless. After cooling, the contents of the flask were washed quantitatively into an Erlnmeyer flask and alkali added until the solution was just distinctly alkaline to litmus. After cooling it was brought back to the acid side and saturated sodium bicarbonate added until the solution was alkaline and 5cc excess was present.

Two cc. of a fresh 1% starch solution was added and the arsenious acid present was titrated with a standard iodine solution, the end point being the characteristic blue of the iodine in the presence of starch. The iodine solution was standardized against standard arsenious acid solution. The latter was prepared from sublimed C.P., As_2O_3. The oxide was dissolved in a neutral solution prepared by the addition of

sodium bicarbonate and a little acid to bring it to neutrality. The arsenious oxide was used as a primary standard and weighed out analytically. The solution was titrated with the iodine solution.

1 cc. of I_2 solution = 0.002186 g of As.

EXPERIMENTAL PART

Preparation of 2, 4 - Dinitrodiphenyl Ether[27].

One hundred grams (.5 mole) of nitrochlorobenzene was added to a solution of potassium phenolate. The latter was prepared by dissolving 94 grams of phenol in 100 cc. of water containing 28 grams of potassium hydroxide. The mixture was then heated under a reflux condenser on an oil bath at 150° for 9 hours. The product was a dark colored solution. Dilute alkali was added to remove the excess phenol and then the ether crystallized out. The supernatant liquid was decanted off and the residue washed several times with water. The crude ether was recrystallized from ethyl alcohol and came down as coarse yellow crystals having a melting point of 70°.

Preparation of 4 - Nitrodiphenyl ether[28].

Seventy grams (1.25 moles) of potassium hydroxide was added to 2 cc. of water and the mixture fused. Seventy grams (1.75 moles) of phenol was added to the potassium hydroxide and the mixture was heated until all the solid had dissolved. One hundred and fifty-seven grams (1 mole)of p-nitrochlorobenzene was added

and the mixture refluxed for about an hour at 200 - 210°. It was then diluted with water and shaken with dilute alkali until cold. A crystalline meal separated out. The supernatant liquid was decanted off and the residue washed with water. The ether was recrystallized from ethyl alcohol and the melting point was found to be 60 - 61°.

Preparation of 2 - Nitrodiphenyl Ether.

This ether was prepared in the same manner as the one described above. Seventy grams of potassium hydroxide was fused with seventy grams of phenol. To this mixture was added 157 grams of o-nitrochlorobenzene. The mixture was refluxed and dilute alkali added to remove the excess phenol. The alkaline layer was separated and the ether was recovered from the other portion by distillation in vacuo.

Preparation of 2 - Amino - 4 - nitrodiphenyl Ether.

One hundred and thirty-six grams (.6 moles) of stannous chloride was added to 240 cc. of ethyl alcohol in a litre flask. The flask and its contents were then weighed. A steady stream of hydrogen chloride gas was bubbled through the mixture until a gain in weight of 56 grams was recorded. During the addition of

hydrogen chloride the stannous chloride went into solution. The alcoholic stannous chloride was then added slowly by means of a dropping funnel to 52 grams (o.1 mole) of 2, 4 - dinitrodiphenyl ether dissolved in 240 cc. of alcohol. During the addition the reaction mixture was stirred rapidly. The temperature was kept below 50 - 60° by cooling the mixture in a bucket of water occasionally. After all the stannous chloride had been added the solution was left standing for an hour to complete the reduction. It was then diluted with a large volume of water and left for several hours. During this time the 2-amino-4- nitrodiphenyl ether separated out as dark red or brown leaflets. It was filtered off and recrystallized from ethyl alcohol. The purified ether was in the form of golden brown crystals. Thirty-two grams of crude amino compound was recovered from 52 grams of the parent material. After crystallizations 10 grams of pure material was left; yield 69% (crude material); m.p. 109.9° Anal. Calcd. for $C_{12}H_{10}N_2O_3$: N, 12.3 Found: N, 11.95 and 11.95.

Preparation of 2 - Aminodiphenyl ether[29].

To a well stirred mixture of 80 grams of zinc dust and 20 grams of calcium chloride in 75 cc. of water and 200 cc. of 95% ethyl alcohol, on a steam bath, was added slowly 43 grams (o.2 mole) of the nitro compound. The reaction was fairly vigorous. After an hour the mixture was filtered to remove zinc oxide and unchanged zinc. The amine was recovered by diluting the solution with water and recrystallized from alcohol; m.p. 44-45°.

Preparation of 4 - Aminodiphenyl ether.

This compound was prepared in a similar manner to the 2- aminodiphenyl ether. Forty-three grams (0.2 mole) of the 4 - nitro compound was reduced by an alcoholic zinc dust mixture and the ether recovered as described above; m.p. 83.5°.

Removal of the Amino Group from 2-Amino-4-nitrodiphenyl Ether.

Four grams of the ether was dissolved in alcohol. To this solution was added 5 cc. of concentrated sulfuric acid. Two grams of sodium nitrite was added and the solution warmed until all the gas (acetaldehyde and nitrogen) ceased coming off. The

solution was diluted with water and an oily tar separated out. It was cooled in the ice box over night and the supernatant liquid decanted off. The residue was recrystallized several times and the melting point determined; m.p. 58°. The melting point of 4-nitrodiphenyl ether was determined as 57°. A mixed melting point of the two compounds was found to be 58°.

Preparation of 3-Nitrodiphenylene Oxide.

Ten grams of 2-amino-4-nitrodiphenyl ether was dissolved in 200 cc. of 6N hydrochloric acid. To this solution was added 6 grams of sodium nitrite, the temperature being kept below 5°. The diazonium solution was poured into a hot solution of 200 cc. of 50% sulfuric acid. The diphenylene oxide was removed from the solution by steam distillation and recrystallized several times from alcohol; m.p.154.3° corr.

Preparation of 2-Nitro-4-arsonodiphenyl ether[31].

Twenty grams of 2-amino-4-nitrodiphenyl ether was dissolved in 400 cc. of HCl (1 conc. HCl:1H_2O) by boiling. The solution was decanted off from the tar formed and cooled. The amine hydrochloride was

diazotized by adding a solution of 10 grams of sodium nitrite in a hundred cc. of water. The sodium nitrite was poured in slowly and the temperature was kept below 5°. The diazonium solution was added to a cold solution of sodium arsenite. The sodium arsenite solution was made up as follows: 50 grams of sodium arsenite and 100 grams of sodium hydroxide was added to 400 cc. of water; 2 grams of copper sulfate was added as a catalyst. Cracked ice was added to keep the temperature down. The mixture was stirred until violent evolution of gas ceased and then allowed to stand over night. It was then warmed and filtered. The filtrate was made acid to litmus with hydrochloric acid and the precipitate that formed filtered off. The precipitate was dissolved in a sodium bicarbonate solution and boiled with charcoal. It was then filtered and acidified. The precipitate was recrystallized from a glacial acetic acid, the arsono compound coming down as brown crystals. This compound does not melt below 250°. Eleven to fourteen grams of crude arsono compound was obtained from twenty grams of the parent compound; yield, 41%.

Anal. Calcd. for $C_{12}H_{11}AsO_4$: As, 25.45.

Found: As, 25.40 and 25.40.

Preparation of 2 - Arsonodiphenyl ether.

This compound was prepared from 20 grams of 2 - nitrodiphenyl ether in the manner described above. It was purified by boiling in a solution of sodium bicarbonate with bone black and recrystallized from acetic acid. It is a white powder definitely crystalline. This compound is more soluble in various solvents (H_2O, acetic acid, etc.,) than the para isomer. The melting point is 175.4 - 177.4° corr.

Anal. calcd. for $C_{12}H_{11}AsO_4$: As, 25.45
Found: As, 25.40 and 25.42.

Preparation of 4 - Amino - 2 - Arsonodiphenyl ether.

Nineteen grams of 4 - nitro - 2 - arsonodiphenyl ether was dissolved in 500 cc. of methyl alcohol and placed in a stoppered flask to facilitate shaking. Two hundred and twenty-five grams of 4% sodium amalgam was added with continual shaking and cooling. The solution was then decanted from the mercury and the methyl alcohol distilled off until a volume of about 50 cc. remained. The solution was then diluted with water and filtered. Excess hydrochloric acid was added to the filtrate which was then allowed to stand for several hours and then it was filtered again.

This removed any excess nitro compound. Sodium hydroxide was then carefully added to the filtrate until a maximum filtrate was formed. The precipitate was filtered off and recrystallized from dilute ethyl alcohol. Five grams of light brown crystals were recovered; yield, 29%.

Anal. Calcd. for $C_{12}H_{12}NAsO_4$: As, 24.21

Found: As, 23.99 and 23.94.

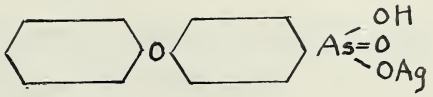
Preparation of the monosilver salt of 4 - Arsonodiphenyl ether.

When the precipitate of 4 - arsonodiphenyl ether was being washed free from chlorides, a solution of silver nitrate was added to the filtrate. A precipitate formed which was dissolved in nitric acid. A quantity of this precipitate was prepared and washed and dried.

Anal. Calcd. for $C_{12}H_{10}O_4AsAg$: Ag, 26.70.

Found: Ag, 26.60.

The structure of this compound is as follows:



Preparation of 2 - Iodo - 4-nitrodiphenyl Ether.

Ten grams of 2 - amino - 4 - nitrodiphenyl ether

was diazotized in the usual way. The diazonium solution was poured into a litre flask and a solution of 25 grams of potassium iodide in 50 cc. water, added. The mixture was allowed to stand for several hours immersed in cold water. Then it was gently heated on a water bath until the evolution of nitrogen ceased. Sodium bisulfite was added to remove the excess iodine. The iodo compound crystallized out. It was filtered and recrystallized several times coming down as white crystals; m.p. 58.6° corr.

Anal. Calcd. for $C_{12}H_8NO_3I$: I, 37.24

Found: I, 37.26.

Preparation of Di(carboxymethyl) phenylthioarsenite.

Three grams (.1 mole) of 4 - arsonodiphenyl ether was dissolved in sodium bicarbonate and 4 grams (0.04 moles) of thiolacetic acid was added. Dilute acetic acid was added until the solution was definitely acid. The derivative came down as a liquid and only crystallized after long standing; m.p. 104.7 - 107.7° corr. The compound was analysed by direct titration with iodine using dilute acetic acid as the solvent.

Anal. Calcd. for $C_{16}H_{15}O_5AsS_2$: As, 17.50

Found: As, 17.10.

PART II

An Investigation of the Bactericidal Power of Some Mono-Mercury Derivatives of Halogen Compounds of Fluorescein.

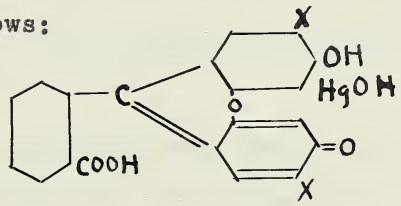
A series of compounds reported in a thesis by S. C. Lynn (University of Alberta) 1932, was investigated. These compounds were:

(1) Monohydroxymercuridichlorofluorescein,

(2) Monohydroxymercuridibromofluorescein,

(3) Monohydroxymercuridi-iodofluorescein.

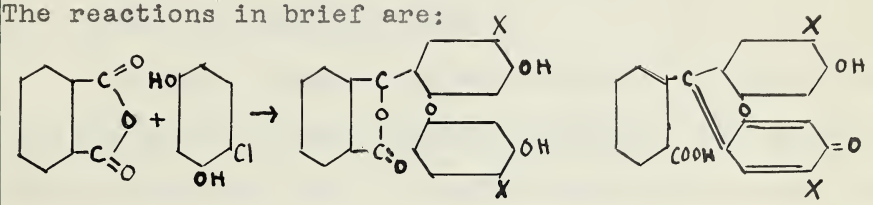
The structure of these compounds may be shown graphically as follows:



where X stands for the halogen substituents.

The chloro compound was prepared from monochlororesorcinol which was obtained by treating resorcinol with sulphuryl chloride. Dichlorofluorescein was prepared by allowing one gram molecule of phthalic anhydride to react with two gram molecules of mono-

chlororesorcinol. The latter was converted to the diacetate and saponified for purification and then heated to 300° transforming the light-colored lactord modification to the darker quinoid modification. The reactions in brief are:



The dichlorofluorescein was mercurated by treatment with mercuric acetate in a solution of glacial acetic acid.

The bromo compound was prepared by the direct bromination of fluorescein to give dibromofluorescein. The latter was then directly mercurated as described above.

The iodo compound was prepared by the direct iodination of fluorescein and subsequent direct mercuration.

It was decided to investigate the bactericidal activities of the three compounds and see how the chemical constitution influenced their activity. The procedure followed in the investigation of the bactericidal action was a modification of the Rideal-Walker method for determining phenol coefficients.

Preparation of Broth.

7.5 g. sodium chloride

7.5 g. Lemco Meat Extract

15 g. Peptone Siccum

1500 cc. distilled water

The above ingredients were mixed in a large flask and autoclaved to get complete solution. The broth was then neutralized with .1N sodium hydroxide and the pH adjusted to 7.2 using bromthymolblue as the indicator. The solution was again autoclaved. The precipitated phosphates were filtered off and the solution put up in 10 cc. portions in 5 x 3/4" broth tubes. After autoclaving the tubes they were ready for use.

Preparation of Solutions of the Fluorescein Derivatives.

A 1 g. portion of the fluorescein derivative was weighed out into a beaker. The theoretical amount or a little in excess of standard sodium hydroxide was added from a burette. The solution was rinsed out into a volumetric flask and made up to 100 cc.

The next step was to prepare the dilutions. The dilutions of 1:100 prepared as above, were used as stock solutions and dilutions were made up immediately

before use. A calculated volume of stock solution was transferred by means of a pipette to a sterilized medication tube. By means of another pipette sterilized water was added to make up the volume of the particular dilution to 5 cc.

Organism.

The test organism was a strain of bacillus typhosus obtained from the Provincial Laboratory. The organism was grown in the broth culture and transferred 24 hours before being used and incubated at 37° C.

Procedure.

A series of 4 dilutions of the disinfectant was placed in plugged sterile medication tubes in 5 cc. portions, for each run. The solution of greatest concentration was placed on the left, the concentration decreasing towards the right. The 5th tube contained a dilution of carbolic acid to serve as a control. In the first runs on the disinfectant a ranging test was made with widely separated dilutions. Then when the killing range was established a series of dilutions was made in this range. Starting at zero time 0.2 cc of the culture was added from a pipette to the left hand medication tube. This tube was flamed and after inoculation the contents were shaken to insure mixing.

The time was checked with a stop-watch. Thirty seconds after the addition the next tube was inoculated and so on until all dilutions were inoculated with the culture of B. typhosus. Thirty seconds from the last addition (i.e., $2\frac{1}{2}$ mins. from zero) a loopful from the first tube was transferred to a flamed broth tube numbered "1". A platinum loop was used. After another 30 second interval a loopful was transferred from the second medication tube to a broth tube numbered "2". This was continued, working left to right until twenty-five broth tubes were inoculated in this manner. The tubes were incubated for 48 hours at 37° C and examined for growth. An opalesence of the broth indicated B. typhosus.

It will be seen that from each dilution loopfuls were withdrawn at intervals of $2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10 and $12\frac{1}{2}$ minutes and placed in the broth.

The platinum loop was flamed before each inoculation to insure complete sterilization. Cotton plugs were kept in all broth tubes and medication tubes, being replaced after all inoculations. The Rideal-Walker method recommends 5 cc. of broth but 10 cc. quantities were used. This was to prevent the bacteriostatic action shown by many disinfectants in low concentrations.

This results in very little or no growth although the organisms are still living. Using 10 cc. portions, the dilution should be great enough to prevent this action.

Results:

The results are summarized as follows, the minimum fatal dose being reported:

(1) Monohydroxymercuridichlorofluorescein:

In dil. 1:1000, growth up to 12½ mins.

In dil. 1:800, growth in 7½ mins., no growth in 10 mins.

In dil. 1:500, growth in 2½ mins., no growth in 5 mins.

(2) Monohydroxymercuridibromofluorescein.

In dil. 1:2000, growth up to 12½ mins.

In dil. 1:1500, growth in 10 mins., no growth in 12½ mins.

In dil. 1:1000, growth in 2½ mins., no growth in 5 mins.

(3) Monohydroxymercuridi-iodofluorescein:

In dil. 1:2000, growth in 10 mins., no growth in 12½ mins.

In dil. 1:1500, growth in 2½ mins., no growth in 5 mins.

In dil. 1:1000 no growth in $2\frac{1}{2}$ mins.

The relative bactericidal activity of the three compounds may be compared by considering their action in a dilution of 1:1000:

(a) Dichloro compound, 1:1000 - growth in $12\frac{1}{2}$ mins.

(b) Dibromo compound, 1:1000 - growth in $2\frac{1}{2}$ mins. but no growth in 5 mins.

(c) Di-iodo compound, 1:1000 - no growth in $2\frac{1}{2}$ min.

It is thus seen the bactericidal action depends on the halogen substituents and increases in the order, chlorine, bromine and iodine, following the order of the elements in the periodic table.

The phenol coefficients of these compounds were not determined since their structure is not similar to phenol. The literature reports the bactericidal power of compounds of this type not in terms of phenol coefficients but in the manner expressed above.

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